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ENHANCED SECRETION OF ELICITINS BY *PHYTOPHTHORA* FUNGI EXPOSED TO PHOSPHONATE

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ABSTRACT - Even though the phosphonate ion drastically reduced the mycelium growth of *Phytophthora cryptogea* and *P. capsici*, it increased considerably the secretion of elicitins (protein elicitors). The enhanced elicitin production, caused by phosphonate, would contribute in activating the plant defences against the fungal aggression.

RÉSUMÉ - La présence de phosphonate provoque une réduction de la croissance du mycélium de *Phytophthora cryptogea* et *P. capsici* et [illegible] augmentation considérable de la secrétion d'élicitines (éliciteurs de nature protéique). Cette production accrue d'élicitines, due au phosphonate, pourrait contribuer à l'activation des mécanismes de défense des plantes contre les attaques fongiques.

MOTS CLÉS: *Phytophthora*, éliciteurs protéiques, phosphonate.

INTRODUCTION

The phosphonate ion (H_2PO_3-), is active against many genera of Oomycetes such as *Phytophthora*, fungal genus represented by numerous species parasitizing diverse types of economically important crops. Phosphonate has a direct inhibitory effect on fungal growth (Bompeix & Saindrenan, 1984; Fenn & Coffey , 1984, 1985; Smillie *et al.*, 1988; Bompeix, 1989; Griffith *et al.*, 1989). It also alters drastically the metabolism of the pathogen, resulting in stimulation of the plant defence mechanisms (Bompeix *et al.*, 1985; Guest, 1986; Saindrenan & Bompeix, 1986; Saindrenan *et al.*, 1988; Guest *et al.*,1989; Dunstan *et al.*, 1990; Nemestothy & Guest, 1990; Ali *et al.*, 1993). The combined effect of pathogen inhibition and of plant defence induction are due to the phosphonate, which finally provides effective and durable control of plant diseases (Guest & Bompeix, 1990). The latter effect involves the potential role of elicitors. It has in fact been shown that phosphonate is responsible for [illegible] significant increase in elicitor glycoconjugates produced by *Phytophthora capsici* (Rouhier *et al.*, 1993).

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Source : MNHN, Paris

Except *Phytophthora parasitica* var. *nicotianae*, all the phytopathogenic *Phytophthora* fungi species, so far studied, secrete large amounts of holoprotein elicitors called elicitins (Pernollet *et al.*, 1993). Elicitins from different species of *Phytophthora*, when applied to tobacco, at equal doses, exhibit different levels of toxicity, but induce protection at the same level (Ricci *et al.*, 1989). They are also toxic to diverse plants other than tobacco (Pernollet *et al.*, 1993; Kamoun *et al.*, 1993; Huet *et al.*, 1994)). Elicitins offer the possibility of precise quantitation in culture filtrates. During the *P. cryptogea* growth, in absence of phosphonate, the amount of secreted elicitin was found to be proportional to the weight of mycelium (Tercé-Laforgue *et al.*, 1992)). We therefore undertook the comparison of the elicitin secretion in presence of phosphonate, using 2 different *Phytophthora* species, *P. cryptogea*, which mainly secretes cryptogein and *P. capsici*, which secretes capsicein.

EXPERIMENTAL

P. cryptogea (isolate 52 from the culture collection of Antibes INRA station and *P. capsici* (isolate 147) were grown in petri dishes, in a standard liquid medium containing asparagine (2g L-1) as the only nitrogen source (Pernollet *et al.*, 1993) or in this medium complemented with phosphonate (3 mM). Studies were conducted in five replicates at 25°C in darkness. Filtrates (20 ml) and mycelia from the 5th and the 16th days were separated by filtration on 0.45 µm filters (Millipore HV type). Mycelia were carefully washed with pure water, dessicated for 24 h before being weighed. The elicitin content of the filtrate was determined from the value of the peak height with reference to purified elicitin calibration after analytical reverse-phase high performance liquid chromatography, as already described (Tercé-Laforgue *et al.*,1992).

RESULTS AND DISCUSSION

Table 1 shows the comparison of the excretion of cryptogein and capsicein in presence and absence of phosphonate. In the absence of phosphonate, the amount of secreted elicitins were proportional to the mycelium growth (data not shown), as already observed in case of *P. cryptogea* (Tercé-Laforgue *et al.*, 1992), and the amount secreted by *P. capsici* was about twice that produced by *P. cryptogea*. Addition of phosphonate to the medium increased its yield by a factor of 8 for cryptogein and 5.5 for capsicein (16th day).

From these experiments we conclude that not only the phosphonate ion affects considerably fungal growth, but that it also causes a drastic increase of the elicitin secretion and/or leakage. Enhanced amount of elicitins, which effectively activates the plant defences (Ricci *et al.*, 1989), would contribute to strengthening the host plant defence against fungal attack. As per our knowledge, this is the first biochemically characterized plant elicitor induced by phosphonate treatment. Since in all earlier instances elicitors were characterized only indirectly. Phosphonate is likely to be a

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Phytophthora capsici				
Time (day)	Mycelium: mg dry wt		Capsicein (µg.mg-1 mycelium dry wt)	
	Control	$H_2PO_3^-$	Control	$H_2PO_3^-$
5	205,6 a	67,6a	5,1a	10,5a
16	359,2b	33,4b*	4,3a	25,7b
Phytophthora cryptogea				
Time (day)	Mycelium: mg dry wt		Cryptogein (µg.mg-1 mycelium dry wt)	
	Control	$H_2PO_3^-$	Control	$H_2PO_3^-$
5	210,2a	7,6a	3,0a	24,2a
16	414,4b	27,4b	1,8a	14,4a

TABLE 1: Mycelium growth and elicitin production in the culture medium in absence and presence of phosphonate (mean of five replicates). * : partial autolysis.
Values with the same letter (within a column) are not significantly different according to Duncanis Multiple Range Test (P= 0,05).

useful tool in order to study the regulation of the elicitin expression at the molecular level in future experiments.

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